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# Food-chain Toxicity of Granular Formulations of Insecticides to a Predator, *Lycosa pseudoannulata*, of *Nephotettix cincticeps*

AUTHOR(S):

KIRITANI, Keizi; KAWAHARA, Sachio

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CITATION:

KIRITANI, Keizi ...[et al.]. Food-chain Toxicity of Granular Formulations of Insecticides to a Predator, *Lycosa pseudoannulata*, of *Nephotettix cincticeps*. 防虫科学 1973, 38(2): 69-75

ISSUE DATE:

1973-05-31

URL:

<http://hdl.handle.net/2433/158793>

RIGHT:

**Food-chain Toxicity of Granular Formulations of Insecticides to a Predator, *Lycosa pseudoannulata*, of *Nephotettix cincticeps*.** Keizi KIRITANI and Sachio KAWAHARA (Division of Entomology, Prefectural Institute of Agricultural and Forest Science, Ino, Kochi) Received January 23, 1973. *Botyu-Kagaku*, 38, 69, 1973.

**10. 粒剤の天敵に対する食物連鎖毒性** 桐谷圭治, 川原幸夫 (高知県農林技術研究所, 高知県吾川郡伊野町) 48. 1. 23受理

ウンカ, ヨコバイ類の捕食性天敵として, 最も重要なキクズキドクグモに対する, 2, 3種ニカメイガ剤の食物連鎖を通じての作用を実験的に調べた。

$\gamma$ -BHC 粒剤では, 土壌-イネ-ツマグロヨコバイ-クモと移行, 濃縮され, 6 kg/10a 施用区では, 1~3週間経過したのちも, このイネを吸汁したツマグロヨコバイは, これを捕食したクモの麻痺, 死亡をもたらす。しかし, cartap, chlorphenamidine 剤の食物連鎖を通じての作用は, 通常の施用条件のもとではみられないが, 施用量が増加すると捕食能力が低下したり, 死亡する可能性が示唆された。

### Introduction

Since 1946, technical BHC together with parathion has been used extensively in Japan to control the rice stem borer, *Chilo suppressalis*. BHC was used initially as an emulsion, but this was followed by a dust, and the dust has been gradually replaced by a granular formulation since 1962.

During this time, resurgence of the green rice leafhopper, *Nephotettix cincticeps*, following the application of BHC dust has commonly been observed in every part of Japan (Kiritani *et al.* 1971)<sup>1)</sup>.

Kobayashi (1961)<sup>2)</sup> claimed that BHC was very toxic to spiders which preyed upon leafhoppers and that annihilation of spiders in BHC-treated paddy fields eventually resulted in resurgence of the leafhoppers. The belief that BHC was no longer toxic to spiders has prevailed over the opinion condemning BHC for its harmful effect, because the emulsion and dust formulations have been replaced by a non-toxic granular formulation. Besides, the persisting high density of the leafhopper was considered to be due to the development of insecticidal resistance in the insect rather than to the elimination of spiders by BHC (Sasaba & Kiritani 1972)<sup>3)</sup>.

Meanwhile, residual effects on wildlife of the organochlorine insecticides, particularly, DDT,

aldrin, and dieldrin, through food chains have been widely recognized all over the world (e.g. Carson 1962<sup>4)</sup>; Rudd 1964<sup>5)</sup>). As regards the food-chain toxicity of chlorinated hydrocarbon insecticides to arthropod predators, there has been published few reports on this respect (van Hateren 1971<sup>6)</sup>), though its occurrence was anticipated theoretically. McClanahan (1967)<sup>7)</sup> was probably the first who demonstrated that the application of systemic acaricides to roots did not provide an ecological selectivity in all cases, and that some materials showed a food-chain toxicity to predacious mites.

As a part of the integrated control programme of rice pests, the food-chain toxicity of  $\gamma$ -BHC to spiders was studied when they were allowed to prey upon leafhoppers that had fed on rice plants grown in a paddy-field water treated with the granular formulation of technical BHC. To compare with BHC, two other insecticides in use for control of *C. suppressalis*, viz. cartap and chlorphenamidine were also examined.

### Methods and Materials

#### Experiment I

Two pairs of 1/50 m<sup>2</sup> pots were transplanted with five rice seedlings at a 4-5 leaf stage. Ten days after transplantation, one pair of the pots received 6%  $\gamma$ -BHC granules at a rate of 3kg/1,000m<sup>2</sup>, and the other one 6kg/1,000m<sup>2</sup> on the

water surface. The water level was kept about 3 cm above the soil surface.

Some thirty adult females of the green rice leafhopper obtained from stock cultures (25°C and with 16 hrs illumination) were caged for 48 hrs in a cylindrical nylon screen cage placed over the rice plants which had been treated with BHC by water surface application. After being fed for 48 hrs on the BHC-treated rice plants, five of these leafhoppers were then introduced together with one adult female wolf spider, *Lycosa pseudoannulata*, into a ½ pint ice-cream cup which contained 2-3 rice seedlings. These cups were maintained at 25°C and 16 hrs illumination. Each spider was fed at intervals of 1-2 days over a period of 10 days with 5 leafhoppers reared successively for 2 days on the same BHC-contaminated rice plants. Thus, the amount of BHC contained in the rice plants on which they were reared might be different depending on the number of days which had elapsed after the application of BHC. The number of replicates was 7 for each dosage group. It should be noted that no leafhoppers appeared to be affected by feeding on the BHC-contaminated rice plants throughout the experiments.

The number of leafhoppers eaten by the spiders and the physical conditions of the spiders, *i.e.* dead, paralysed and living, were daily examined and recorded.

#### Experiment II

This experiment is almost similar to Exp. I, but differed in that the spiders were fed daily with leafhoppers reared on the rice plants after the same number of days of insecticidal treatment, *i.e.* 0, 3, 7, 10 or 12 and 15 or 18 days, whereas in Exp. I the spiders were fed with leafhoppers which differed in the number of days after the insecticidal treatment as the experiment went on.

The insecticides used were granular type of formulations of 6%  $\gamma$ -BHC, 4% cartap and 3% chlorphenamidine. Spiders were fed daily with 5 leafhoppers reared for 2 days on rice plants after the same number of days of insecticidal treatment. Spiders fed with the leafhoppers taken directly from the stock cultures served as controls. All the experiments were conducted at 25°C

and with 16 hrs illumination. The total numbers of spiders used for this experiment were 130 and 65 for treatments and controls, respectively.

#### Experiment III

Thirty four pots of 1/50 m<sup>2</sup> containing paddy soils were prepared and 5-6 seedlings bearing 5-6 leaves each was planted in each pot. The water surface was kept 3-5 cm above the soil throughout the experiment. After 20 days, *i.e.* at the tillering stage, 6%  $\gamma$ -BHC granules were applied to the water surface at a rate of 3 kg/1,000m<sup>2</sup>.

Twenty of the pots was divided into two groups; one group of 13 pots were for analysis of the BHC residues in soil and rice plants; the other group of 7 pots were for analysis of the residues in the leafhoppers which had fed on the BHC-contaminated rice plants. Three days after the BHC application, the soils of top 5 cm were collected and air dried, while rice plants were cut at their base above the soil and were kept in a deepfreezer at -20°C. The other group, which consisted of 7 pots, was infected with some 40 leafhoppers per pot for 2 days (a total of 300 leafhoppers) and then frozen at -20°C. 560 leafhoppers obtained from the stock cultures acted as a control.

Spider samples were prepared with 14 pots in a similar way to Exp. II, *i.e.* so as to feed the spiders with leafhoppers reared for 2 days on rice plants which had received BHC-granules 3 days before caging the leafhoppers. Each spider was fed with BHC-contaminated leafhoppers at a rate of 5 insects per day for up to 7 days. Ten spiders were used in all. Some of these died during the experiment and these were placed immediately in the freezer. Before storing the spiders, physiological states of individual spiders, *i.e.* dead, paralysed and living, were recorded. A sample of 10 spiders collected from a paddy field served as a control.

#### Analytical method

**Soils and rice plants:** Twenty gram of air-dried soil was blended with 100 ml of acetonitrile water (7:3) and then centrifuged for 5 minutes. Fifty ml of the supernatant was shaken with *n*-hexane, and the hexane layer passed through a Florisil

column, was concentrated to 5 ml. The concentrate was analysed by a gas-liquid chromatography (Shimadzu GC 5A type gas chromatograph equipped with an electron capture detector) using OV-17 and DEGS- $\text{H}_3\text{PO}_4$  columns.

Samples of the rice plant were ground with water and were extracted with acetonitrile. Thereafter, the procedures employed were the same as with the soil samples.

**Leafhoppers and spiders:** For analysis the specimens were extracted three times in the presence of 1 gr of anhydrous sodium sulphate, in a waring blender for 5 minutes with 10 ml-portions of *n*-hexane. The combined hexane extract was centrifuged for 10 minutes, and the resulting supernatant was cleaned through a Florisil column, and then concentrated to 5 ml. The BHC isomers in samples were analysed by gas-liquid chromatography using an OV-17 column.

## Results and Discussion

### Expt. I

Some spiders died or temporarily paralysed when they were fed with leafhoppers which had fed on the BHC-contaminated rice plants. The deeply paralysed ones laid on their back trembling, but they resumed normal activity after one or two days. Obviously, there was a food-chain toxicity of BHC to spiders through the BHC-contaminated prey.

No spiders died when they fed on prey derived from plants receiving 3 kg/1,000 m<sup>2</sup> and only one individual out of 7 was paralysed. This individual had been fed with the leafhoppers reared for 2 days on the rice plants 9 days after BHC treatment (hereafter this treatment is referred to as the 9th day). Contrary to this, one individual was paralysed as early as on the 4th day, and dead individuals were observed on the 6th day, among spiders fed with prey from the plants receiving 6 kg/1,000 m<sup>2</sup>. The cumulative mortality amounted to 28%, and dead and paralysed individuals made up 57% of the total by the end of the experiment, *i.e.* 10 days.

### Expt. II

Since Expt. I clearly showed that there was a food-chain toxicity of BHC granules through rice plants and leafhoppers to spiders, the food-chain

toxicity of two other granular formulations of insecticides which were widely used for the control of *C. suppressalis* were also examined for comparison.

The results of Expt. II are shown in Table 1. Since we established controls for each treatment of the different insecticides, we examined the mortality and daily rate of predation for a total of 65 spiders which were fed with uncontaminated leafhoppers during a period of 10 days. Since no individuals were affected and there was little variation in the daily predation rate between the controls, records for the control spiders were pooled: mortality was 0% and the daily rate of predation was  $3.38 \pm 0.06$  (mean  $\pm$  95% fiducial limit). The daily rate of predation by spiders was obtained by dividing the total number of prey eaten by the number of days during which they were living, including paralysis.

### 6% $\gamma$ -BHC granules

The food-chain toxicity to spiders was evident even for those spiders which were fed with 0th-day leafhoppers which fed on rice plants for 2 days immediately after the BHC treatment, and this suggested that intake of BHC through rice plants and leafhoppers to spiders occurred within a couple of days after the BHC treatment. In both series of 3 kg and 6 kg/1,000 m<sup>2</sup>, the effect of the food-chain toxicity to spiders was very great when they were fed with 3rd- and 7th-day leafhoppers. Five out of 6 individuals were killed within 3 days after the beginning of the feeding experiment in the group of 6 kg/1,000 m<sup>2</sup> (Fig. 1). This indicated that the concentration of BHC in the rice juice reached to a maximum several days after its application.

Reduction in the daily predation rate was observed in every treatment, but there was a short delay in its occurrence. The greatest reduction in the rate was observed in the spiders fed with 7th- and 12th-day leafhoppers.

Dempster (1967)<sup>8)</sup> observed that DDT markedly reduced the rate of feeding by adult *Harpalus rufipes* at significantly lower concentrations than that required to kill them. This effect of the organochlorine insecticides is likely to be widespread among other species of arthropod predators. It is worthy to mention that even leafhoppers that

Table 1. The effect of granular formulation of three insecticides on *Lycosa* spiders through the food-chain.

Insecticides and application rates	Items	Days after treatment <sup>1)</sup>						
		0	3	7	10	12	15	18
6% $\gamma$ -BHC 3kg/1, 000m <sup>2</sup>	Alive	2	0	1	—	1	—	2
	Paralysed	1	1	0	—	2	—	0
	Dead	0	2	2	—	0	—	1
	Daily no. of prey eaten <sup>2)</sup>	2.50±0.68 <sup>3)</sup>	3.27±0.73	3.10±0.38	—	2.56±0.29	—	4.13±0.27
6kg/1, 000m <sup>2</sup>	Alive	0	0	0	—	0	—	0
	Paralysed	1	0	0	—	3	—	0
	Dead	2	3	3	—	0	—	3
	Daily no. of prey eaten <sup>2)</sup>	3.20±0.45	3.17±0.17	1.83±0.60	—	1.97±0.18	—	3.46±0.43
3% chloropne- namidine 3kg/1, 000m <sup>2</sup>	Alive	5	5	5	5	—	5	—
	Paralysed	0	0	0	0	—	0	—
	Dead	0	0	0	0	—	0	—
	Daily no. of prey eaten <sup>2)</sup>	3.50±0.13	3.56±0.11	2.98±0.08	3.18±0.11	—	3.22±0.13	—
6kg/1, 000m <sup>2</sup>	Alive	5	4	5	5	—	4	—
	Paralysed	0	0	0	0	—	1	—
	Dead	0	1	0	0	—	0	—
	Daily no. of prey eaten <sup>2)</sup>	3.52±0.08	3.30±0.17	3.04±0.13	3.50±0.08	—	3.00±0.17	—
4% Cartap 3kg/1, 000m <sup>2</sup>	Alive	5	5	5	5	—	5	—
	Paralysed	0	0	0	0	—	0	—
	Dead	0	0	0	0	—	0	—
	Daily no. of prey eaten <sup>2)</sup>	2.66±0.16	2.94±0.22	2.80±0.24	3.45±0.31	—	2.82±0.55	—
6kg/1, 000m <sup>2</sup>	Alive	5	5	5	5	—	5	—
	Paralysed	0	0	0	0	—	0	—
	Dead	0	0	0	0	—	0	—
	Daily no. of prey eaten <sup>2)</sup>	2.82±0.26	2.82±0.24	3.20±0.17	3.52±0.25	—	2.85±0.36	—

<sup>1)</sup> See text for further details<sup>2)</sup> The daily number of prey eaten per spider for 65 control spiders was 3.38±0.06<sup>3)</sup> Mean±S. E. (95% fiducial limit)

reared on rice plants 18 days after the BHC treatment still contained a BHC concentration that was high enough to kill spiders.

### 3% Chlorphenamidine granules

No spiders were affected by the treatment of 3% chlorphenamidine at a rate of 3 kg/1,000m<sup>2</sup>. Although the daily rate of predation remained normal compared with control spiders, some

individuals were affected in the series of 6 kg/1,000m<sup>2</sup>. In this series, one individual fed on 15th-day leafhoppers showed symptoms of paralysis 4 days after the start of feeding experiment, and this spider died on the 13th day, 3 days after the end of feeding experiment. In all, 3 out of 25 in the series of 6 kg/1,000m<sup>2</sup> died within 5 days after the end of feeding experiment,

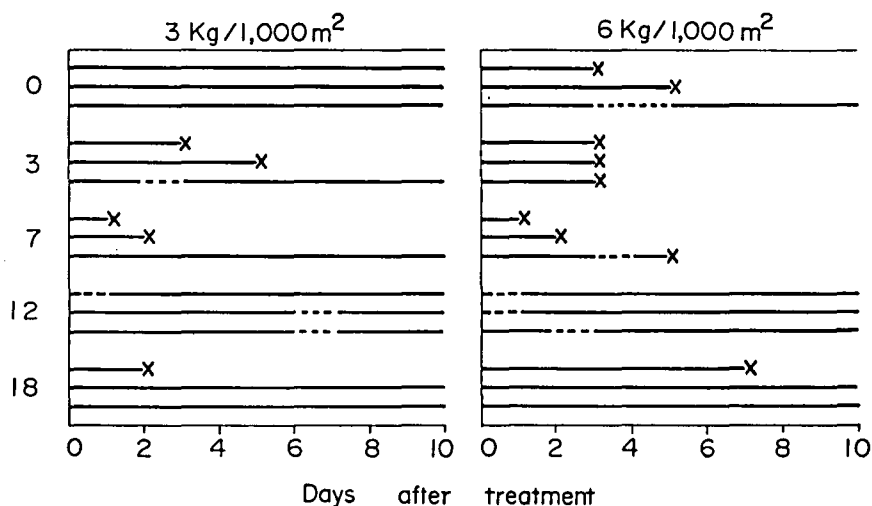


Fig. 1. Daily records of individual spiders fed with BHC-contaminated leafhoppers. Leafhoppers were reared for 2 days on rice plants which were treated with 6%  $\gamma$ -BHC granules at different rates, i.e. 3kg and 6kg/1,000m<sup>2</sup>. Ordinates indicate the number of days which had elapsed after the BHC treatment of rice plants on which leafhoppers were reared. The experiments were suspended after 10 days. —: living, .....: paralysed and x: dead.

although they were fed with uncontaminated prey after the end of experiment.

#### 4% Cartap granules

No appreciable effect was observed with either rate of cartap application, i.e. 3 kg or 6 kg/1,000m<sup>2</sup>. With regard to the predation rate, however, spiders fed with 0th- and 3rd-day leafhoppers showed a slight decrease in their predation rates as compared with control one. This decrease was observed regardless of the amount of chemical applied.

#### Expt. III

This experiment attempted to trace the uptake of BHC and to prove that BHC itself was responsible for the death of spiders. The results of analysis for BHC residues at various trophic levels are shown in Table 2. Several points of interest will be drawn from this table.

First, it appears that there was no biological concentration of BHC through the food chain at the predator level (see Moriarty, 1972<sup>9),10</sup>). Leafhoppers contained 3 times as much total BHC as that in rice plants after 2-day feeding on the BHC-contaminated plants. Added to this, the proportion of  $\gamma$ -BHC in total BHC increased

stepwise through soil, plant and leafhopper, until it made up 35% of the total BHC in the leafhoppers. The fact that leafhoppers concentrate BHC in their body and particularly  $\gamma$ -BHC selectively is significant because this isomer is exceptionally toxic to spiders, the predator of leafhoppers. It is known that *Lycosa* is very susceptible to  $\gamma$ -BHC and the relative toxicity of  $\gamma$ -BHC expressed in LC<sub>50</sub> to *Lycosa*/LC<sub>50</sub> to leafhoppers is only 0.02<sup>11</sup>).

Secondly, the concentration of total BHC was highest in living spiders followed by the paralysed and dead in decreasing order. The concentration of  $\gamma$ -BHC in dead spiders amounted to 5 times that in survived ones. This might suggest that degradation of  $\gamma$ -BHC occurred efficiently in survived spiders, while dead ones failed to decompose  $\gamma$ -BHC before it had become lethal. This point, however, await further study. The dead spiders may have eaten more highly contaminated leafhoppers, since we have no information about variations in the residues in individual leafhoppers. Alternatively, the concentration of BHC in spiders was measured on the basis of their wet weight, so that the dead ones would

Table 2. BHC residues in different trophic levels. Residues in the soil and in the plants were examined 3 days after the water surface application of 6%  $\gamma$ -BHC granules<sup>1)</sup> at a rate of 3 kg/1,000m<sup>2</sup>. The residues in leafhoppers were examined after a 2-day feeding on these rice plants. Spiders used for residue examination were fed with these leafhoppers for 10 days at most (see text for further details).

Items		No. of leafhoppers or spiders used	Detected amounts (ppm)					Isomer ratio (%)				
			$\alpha$	$\beta$	$r$	$\delta$	Total	$\alpha$	$\beta$	$r$	$\delta$	
Treated	soil	—	4.025	0.651	0.772	0.411	5.859	68.7	11.1	13.2	7.0	
	rice plant	—	1.826	0.384	1.504	1.435	5.149	35.5	7.5	29.2	27.9	
	leafhopper	300	7.908	0.763	5.339	1.246	15.256	51.8	5.0	35.0	8.2	
	spider	living	4	0.806	0.278	0.025	0.008	1.117	72.2	24.9	2.2	0.7
		paralysed	3	0.827	0.181	0.022	0.008	1.038	79.7	17.4	2.1	0.8
		dead	3	0.585	0.097	0.126	0.019	0.827	70.7	11.7	15.2	2.3
Control	BHC granule (5.5% $\gamma$ -BHC)							65.2	14.2	15.6	5.0	
	leafhopper	560	0.066	0.027	0.077	0.006	0.176	37.5	15.3	43.8	3.4	
	spider living	5	0.053	0.022	0.025	0.007	0.107	49.5	20.0	23.4	6.5	

<sup>1)</sup> The exact content of  $\gamma$ -BHC was 5.5%

likely have lost water and this would have increased the apparent concentration of  $\gamma$ -BHC. Horiguchi (1970)<sup>12)</sup> also observed that the amount of residues was greater in dead insects than in living ones when *Oulema oryzae* was treated with  $\gamma$ -BHC. The fact that the amounts of  $\beta$ -BHC in the living and paralysed ones were 2-3 times that in dead ones is worthy to note, because of the four BHC isomers only  $\beta$ -BHC is the principal pollutant of natural environments including living animals (Kiritani 1971<sup>13)</sup>). From the viewpoint of the biological concentration of persistent chemicals through food chains, the amount of residues in living animals is much more significant than those in dead, because survived ones with higher  $\beta$ -BHC concentrations may further be concentrated through food chains in the ecosystem.

Thirdly, detection of BHC in control animals was surprising, particularly in the leafhoppers from stock cultures, since they were transferred directly from cultures to freezer. The sources of contamination was unknown. This may indicate, however, that this level of BHC is a sort of background concentration in the environment of

Japan as a result of a wide use of technical BHC.

### Conclusion

These experiments clearly demonstrated the persistence of activity of soil-applied BHC in killing lycosid spiders, *Lycosa pseudoannulata*, through the food chain, namely, from soil or irrigated water, through rice plants and leafhoppers to spiders. This finding provided a scientific basis for the voluntary restraint in the use of BHC for *C. suppressalis* in Kochi Prefecture in 1969, two years before the legal ban on BHC in Japan.

In association with BHC granules, the same formulation of chlorphenamidine and cartap have been used for the control of *C. suppressalis*. In order to replace BHC with other alternative insecticides which have an ecological selectivity, we examined the possibility of the food-chain toxicity of two candidate chemicals, *i. e.* chlorphenamidine and cartap. The results suggest that these are satisfactorily safe when they are used at the minimum commercial rate, *i. e.* 3 kg/1,000 m<sup>2</sup>.

**Acknowledgement** We wish to thank Mr. M. Uyeta, Kochi Prefectural Public Health Laboratory for analysing the leafhoppers and spiders, and Mr. K. Yamamoto, Laboratory for Pesticide Residues, Kochi Pref. Inst. Agric. & Forest Science, for analysing the soils and rice plants for BHC residues. Thanks are also due to Drs. J. P. Dempster, Monks wood Experimental Station, England; M. Chiba, Vineland Research Station, Canada Department of Agriculture; and C. Hirano, Kochi University, Japan.

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**Metabolism of Pyridafenthion, O, O-Diethyl-O-(3-oxo-2-phenyl-2H-pyridazin-6-yl) phosphorothioate, in Mouse and Rat.** Takatoshi UDAGAWA, Tetsuo SAITO and Tadashi MIYATA (Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya, Japan) Received December 12, 1972. *Botyu-Kagaku*, **38**, 75, 1973. (with English Summary 81)

**11. Pyridafenthion, O, O-Diethyl-O-(3-oxo-2-phenyl-2H-pyridazin-6-yl) phosphorothioate のマウスおよびラットにおける代謝** 宇田川隆敏, 斎藤哲夫, 宮田 正 (名古屋大学農学部害虫学教室, 名古屋市中千種区不老町) 47. 12. 12 受理

<sup>32</sup>P- および 3,6-pyridazine-<sup>14</sup>C-pyridafenthion をマウスおよびラットに経口投与し、一定時間毎に尿への排泄量、24時間後における糞、各種臓器における放射能を調べた。いずれの場合も急速に代謝され24時間後には <sup>32</sup>P-fenitrothion と同様に投与量の70%以上が尿に分解物として排泄された。3週間、100ppm の薬剤を連続投与しても <sup>32</sup>P-, <sup>14</sup>C-pyridafenthion の代謝排泄は <sup>32</sup>P-fenitrothion の連続投与の場合と同様変化はなかった。投与薬剤の大部分は尿に水溶性物質として排泄され、ラットでは phenyl maleic hydrazide と desethyl-pyridafenthion-oxon、マウスでは phenyl maleic hydrazide とその glucuronide であった。従って pyridafenthion のマウス、ラットにおける主要な分解は P-O-aryl 開裂であり、<sup>14</sup>C-phenyl maleic hydrazide は <sup>32</sup>P-O, O-diethyl phosphorothioic acid と同様に急速に排泄された。

有機リン化合物はすぐれた殺虫力と高等動物に対する低い毒性、短い残留性から有機塩素系殺虫剤にかわって、近年代表的殺虫剤群となり、各種の化合物が実用されてきている。これらの化合物はいずれも五価のリンを中心としたもので、これに結合するアルキル基やアリル基等の置換によりその特性は著しくかわる。

本報では新規有機リン殺虫剤である pyridafenthion, O, O-diethyl-O-(3-oxo-2-phenyl-2H-pyridazin-6-yl) phosphorothioate、について <sup>32</sup>P または <sup>14</sup>C で標識し、マウスやラットにおける代謝を調べたので報告する。